BIO-RAD

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## SUMMARY OF SAFETY AND EFFECTIVENESS

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Product Trade Name: VARIANT Alpha-Thalassemia Short Program

Common Name: VARIANT Hemoglobin Testing System

Classification Name: Abnormal Hemoglobin Assay, 81LGL

The VARIANT Alpha-Thalassemia Short Program is designed for use on the fully automated VARIANT analyzer. The analytical system consisting of instrument and reagent kit provides an assay for the detection of hemoglobin Bart's by High Performance Liquid Chromatography (HPLC).

To establish substantial equivalence to an existing device, and thus establish the safety and effectiveness of the VARIANT Alpha-Thalassemia Short Program, the program has been compared to the Isolab Alpha-Thal Screen test. A review of the intended use of each system shows them to be essentially the same. The intended use of the VARIANT Alpha-Thalassemia Short Program is stated: The determination of hemoglobin Bart's in human whole blood using ion-exchange HPLC. The intended use of the Isolab Alpha-Thal Screen test is stated as: Accurate quantitation of Hemoglobin Bart's in cord blood as an aid to diagnosis of Alpha-Thalassemia.

The VARIANT Alpha-Thalassemia Short Program utilizes the principles of cation exchange HPLC. A hemolysate is prepared by mixing whole blood with a hemolysis reagent included in the kit. The prepared hemolysate is placed into the instrument's auto sampler. The sampler injects a specified amount of hemolysate onto the cartridge. The separation of normal hemoglobin and Hb Bart's is accomplished on the cation exchange cartridge using a buffer gradient. A dual wavelength photometer (415 and 690 nm) monitors the elution of the separated hemoglobins from the cartridge, detecting absorbance changes at 415nm. The 690 nm secondary filter corrects the baseline for effects caused by the mixing of buffers of different ionic strengths as the gradient is formed.

In the Variant α-Thalassemia Program, Retention Time Windows have been established for the quantitation of Hb Bart's and all non-Bart's hemoglobins, except Hb S and Hb C. These "windows" are expressed in time (minutes and fractions thereof) called retention time. Retention time relates to how long a peak takes from the point of injection to the apex of the peak representing a given hemoglobin. In the Variant α-Thalassemia Program, if a peak occurs within one of these preset windows, it is designated as the hemoglobin corresponding to that window. For example, if a peak falls in the "Bart's window" the report indicates that a peak was detected in the "Bart's window" and prints a quantitative value on the sample report.

For quantitation, a calibrator, with an assigned Hb Bart's value which is entered into the software during setup of the assay, is placed at the beginning of a run. After the analysis of the calibrator, a calibration factor is determined from the ratio of the assigned value to the observed value. This calibration factor is applied to each subsequent sample in the run.

The Isolab Alpha-Thal Screen test utilizes open column liquid chromatography to separate the hemoglobins in the prepared hemolysates. In this test, a hemolysate of whole cord blood is absorbed onto a preconditioned CN-52 cellulose column bed. Hb Bart's is selectively eluted from the column under specific conditions of pH and chloride concentration. Subsequently the non Hb Bart's fraction is eluted from the column. The % Hb Bart's is determined by measurement of the absorption of both fractions at 415nm.

The performance of the VARIANT Alpha-Thalassemia Short Program was evaluated for precision, accuracy, and linearity and recovery. Precision studies were done according to NCCLS vol. 12 No 4, EPS-T2. Within-Run Precision values were: Low % Hb Bart's SD 0.0 and CV 0.0; Medium % Hb Bart's, SD 0.1 and CV 1.6; High % Hb Bart's SD, 0.2 and CV 1.1. Day to Day Precision Values were: Low % Hb Bart's SD 0.1 and CV 74.0; Medium % Hb Bart's, SD 0.2 and CV 3.2; High % Hb Bart's, SD 0.7 and CV 3.3.

Accuracy was determined by a correlation study against the Isolab Alpha-Thal Screen test the results of which are shown in Appendix L. 195 cord blood specimens, including normal, Alpha-Thal 1, Alpha-Thal 2, and Hb H disease patients were analyzed on the VARIANT Alpha-Thalassemia Short and the ISOLAB "Alpha-Thal Screen." Statistical comparison of the Hb Bart's values obtained on the VARIANT Alpha-Thalassemia Short and the ISOLAB "Alpha-Thal Screen" yielded a correlation coefficient of 0.977, a y-intercept of 0.79 and a slope of 1.2.

The linearity study was performed to evaluate the response of the VARIANT to increasing concentrations of Hb Bart's. In this study a hydrops sample, which was hemolysed in the Alpha-Thal Short Hemolysis Reagent, was mixed with a normal sample and analyzed. The measured results from the VARIANT were compared to the theoretical values calculated from the ratio's Hydrops/Normal patient sample. The linearity results yielded a correlation coefficient of 1.0, a y-intercept of -0.51 and a slope of 0.99.

It can be concluded from the correlation study between the Bio-Rad Variant Alpha-Thal Screen test, and the similarities of the general characteristics, that the two assays are substantially equivalent. Based on the establishment of substantial equivalence, the safety and effectiveness of the Bio-Rad VARIANT Alpha-Thalassemia Short Program is confirmed.